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SODIUM BISULFITE

TOXICITY and TERATOGENICITY STUDIES in Avian Embryos

FDA Contract #71-330

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STUDIES on the TOXICITY and TERATOGENICITY of SODIUM BISULFITE in AVIAN EMBRYOS

SUMMARY and CONCLUSIONS

Sodium bisulfite was embryo toxic when administered in either the air cell or yolk of fertilized chicken eggs at either zero or 96 hours incubation. This compound was more toxic when injected into the air cell than when the yolk administration route was employed.

The incidences of abnormalities in these tests were not significantly higher than was noted for the solvent controls. Sodium bisulfite was not teratogenic under the conditions of these studies.

GENERAL PROCEDURES

The protocols as specified under FDA Contract #71-330 were followed in the investigation of toxicity and potential teratogenicity of the specified substance. The toxicity of the substance was evaluated from the percentage hatch of embryos injected either in the air cell or yolk at either zero hours (post-incubation) or after 96 hours incubation to provide four separate evaluations.

EGG SOURCE AND HANDLING

All eggs used in these investigations were from Shaver Starcross pullets housed at the Poultry Research Center of the University of Arizona in Tucson. The parent stock was maintained on the University of Arizona breeder diet which had been formulated to provide more than adequate amounts of all the known nutrients required by the breeding hen.

The feed was specially prepared to assure no contaminations and did not contain any additive drugs such as antibiotics. All eggs prior to use (within 48 hours of lay) were candled to remove any containing blood spots, abnormal air cells or abnormal shells, and only clean eggs ranging in weight from 23 - 26 ounces per dozen were used.

The supply flock was tested to assure the absence of Pullorum and Mycoplasma gallisepticum.

The eggs were incubated in forced draft Jamesway 252 machines with automatic temperature and humidity controls and an automatic turning device.

COMPOUND HANDLING FOR INJECTION

The substance tested was solubilized in a number of the prescribed solvents in order to determine the maximum concentrations which could be employed. Where possible, water was the solvent of choice. Maximum

injection volume was 0.05 ml. and all solvents and glassware were autoclaved prior to preparation of the solutions for use. The dose levels were administered with a microliter syringe using sterilized needles.

The preliminary range-finding studies using each of the administration routes and times were carried out with 10 - 25 eggs per dose level and included solvent controls, untreated controls and either drilled or pierced controls.

The actual dose-response protocol was carried out in two or more injections on different days to produce a minimum of 100 eggs at each dose level in five or more levels selected from the range- finding studies.

EXAMINATIONS OF EMBRYOS AND CHICKS

Eggs were candled daily and the dead embryos removed, examined and any abnormalities recorded. Five chicks from each dose level in each hatch were X-rayed to determine any skeletal abnormalities. Additional eggs injected at the approximate LD-50 level and an additional level below that were incubated and embryos at 8, 14, 17 days and hatch chicks removed for histopathological examinations.

In additional studies representative chicks from the dose-response protocol were saved. These chicks were housed in electrically-heated battery brooders with raised wire floors and fed University of Arizona diets. Feed consumption and growth rates were evaluated at 6 weeks of age and a sample of the birds sacrificed for gross and histopathological examinations.

The remaining birds in each group were maintained to 6 months of age and then sacrificed.

DATA HANDLING

All data were coded on forms provided by FDA for computer input. In addition to summaries of mortalities and abnormalities, a number of statistical evaluations were carried out. These statistical analyses included the following for both mortality and the incidence of abnormal embryos:

1. Chi-square tests for all dose levels and for each level against the solvent control.
2. Linear regression analyses + chi square test of linearity.
   a. % response against dose
   b. % response against log dose
   c. log % response against dose
   d. arcsin transformation against dose
   e. arcsin transformation against log dose
3. Log dose against Probit using Finney's maximum likelihood method.
   a. Where significant, the LD-30, 50, 70 and 90's were estimated with 95% confidence intervals.
4. One-way analyses of variance.
5. Linear regression with replication.

Sodium bisulfite (71-20) was solublilized in water for use in the test protocols. The maximum dose level employed was attained with a solution of 200 mg/ml to provide the 200 mg per kg (10 mg/egg).

MORTALITY

The mortality data for the individual test protocols are shown in Tables 1 - 4. The dose ranges employed in these studies produced an upper level of 100% mortality in the air cell series and 85 - 95% mortality with yolk administrations. Statistical evaluations employing the linear regression analysis program of log dose against probit indicated a significant relationship for all but the yolk - 0 hour series; while chi-square evaluations showed significantly higher ($P>0.05$) mortality in comparison with the solvent controls in each series (Table 5). Statistical estimates of LD50 for the three protocols where significant relationships were obtained are shown in Table 6. These data suggest that the LD50 dose varies with administration route and time of injection with an estimate of 9.7 mg/kg for air cell - 0 hours and 121.3 mg/kg for yolk injection at 96 hours.

Sodium bisulfite is highly embryo toxic in chickens when administered in either air cell or yolk at either zero or 96 hours incubation.

TERATOLOGY

The incidence of abnormalities in these studies was quite low in the groups receiving sodium bisulfite (Tables 1 - 4). Chi-square analyses of the data on incidence of abnormalities showed no statistical significance ($P\leq 0.05$) for any of the test protocols (Table 7). The

other statistical evaluations also failed to detect a significant increase in abnormalities in embryos exposed to sodium bisulfite in comparison with the solvent-treated controls. Chi-square analyses of head-limb-skeletal-viscera abnormalities (excluding toxic tesponse and functional abnormalities) were also not statistically significant at the 0.05 level of probability (Table 8). The teratology findings are reported in Table 9.

POST-HATCH DATA

The chicks maintained for six months post-hatch showed no significant alterations in growth rate, feed consumptions or sexual maturity in comparison with the solvent controls (Table 10).

TABLE 1

Sodium Bisulfite in H_2O
Air Cell - 0 Hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functiona %	Functiona #
00.0	113	100.00	113	0.00	0	0.00	0														
00.0	119	98.31	117	0.00	0	0.00	0														
50.0	18	100.0	18	5.55	1	5.55	1			5.55	1										
25.0	97	81.44	79	0.00	0	0.00	0														
20.0	120	60.00	72	0.00	0	0.00	0														
10.0	114	60.52	69	0.00	0	0.00	0														
5.0	136	39.70	54	0.00	0	0.00	0														
4.0	99	37.37	37	4.04	4	3.03	3	2.02	2			1.01	1					1.01	1		
2.0	99	21.21	21	2.02	2	2.02	2	1.01	1					1.01	1						
0.0	241	10.78	26	0.00	0	0.00	0														
rilled	175	14.85	26	0.00	0	0.00	0														
ontrol	663	5.88	39	1.05	7	0.60	4	0.45	3			0.15	1			0.30	2			0.30	

SUMMARY - ALL DOSE LEVELS

915	63.39	580	0.77	7	0.66	6	0.33	3	0.11	1	0.11	1	0.11	1	0.00	0	0.11	1	0.00	

TABLE 2

Sodium Bisulfite in H_2O
Air Cell - 96 Hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functiona %	Functiona #
100.0	59	100.00	59	0.00	0	0.00	0														
40.0	119	100.00	119	0.00	0	0.00	0														
30.0	116	94.82	110	0.00	0	0.00	0														
25.0	56	87.50	49	0.00	0	0.00	0														
20.0	216	55.09	119	0.00	0	0.00	0														
10.0	227	44.05	100	0.00	0	0.00	0														
5.0	175	14.85	26	0.00	0	0.00	0														
0.0	224	7.14	16	0.00	0	0.00	0														
drilled	264	5.30	14	0.37	1	0.37	1	0.37	1												
control	663	5.88	39	1.05	7	0.60	4	0.45	3			0.15	1			0.30	2			0.30	

SUMMARY - ALL DOSE LEVELS

968	60.12	582	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	

TABLE 3

Sodium Bisulfite in H_2O
Yolk - 0 Hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functio %
200.0	100	95.00	95	0.00	0	0.00	0													
100.0	102	92.15	94	0.00	0	0.00	0													
25.0	101	87.12	88	1.98	2	0.00	0									0.99	1	0.99	1	
10.0	100	80.00	80	1.00	1	2.00	2	1.00	1					1.00	1					
5.0	97	78.35	76	1.03	1	1.03	1	1.03	1											
0.0	98	17.34	17	1.02	1	1.02	1	1.02	1											
pierced	152	43.42	66	0.00	0	0.00	0													
control	663	5.88	39	1.05	7	0.60	4	0.45	3			0.15	1			0.30	2			0.30

SUMMARY - ALL DOSE LEVELS

500	86.60	433	0.80	4	0.60	3	0.40	2	0.00	0	0.00	0	0.20	1	0.20	1	0.20	1	0.00

TABLE 4

Sodium Bidulfite in H_2O
Yolk - 96 Hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functio %
200.0	99	80.80	80	0.00	0	0.00	0													
100.0	99	48.48	48	0.00	0	0.00	0													
40.0	100	20.00	20	0.00	0	0.00	0													
4.0	100	20.00	20	0.00	0	0.00	0													
1.0	98	16.32	16	0.00	0	0.00	0													
0.0	230	14.78	34	0.43	1	0.43	1	0.43	1											
pierced	118	10.16	12	1.69	2	1.69	2	1.69	2											
control	663	5.88	39	1.05	7	0.60	4	0.45	3			0.15	1			0.30	2			0.30

SUMMARY - ALL DOSE LEVELS

496	37.10	184	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00

TABLE 5

SODIUM BI-SULFITE
CHI-SQUARE ANALYSES of MORTALITY

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
1.0	-	-	-	0.04
2.0	5.56*	-	-	- -
4.0	31.12*	-	-	1.03
5.0	41.78*	5.42*	70.30*	-
10.0	95.16*	78.48*	75.26*	- -
20.0	95.63*	116.63*	-	-
25.0	157.95*	157.81*	94.40*	-
30.0	-	248.17*	-	- -
40.0	-	276.88*	-	1.03
50.0	88.30*	-	-	-
100.0	251.27*	201.99*	110.25*	40.23*
200.0	253.01*	-	118.34*	130.34
All Doses (DF)	481.88* (9)	599.21* (7)	222.39* (5)	181.32* (5)

*Probability > 0.05 - 0.005.

TABLE 6

PROBIT ANALYSES
(Log Dose against Probit of Mortality)

	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
LD-30	4.8	9.6	NS	86.0
(Range)	(3 - 6.6)	(5.7 - 12.6)		(56.4 - 107.2)
LD-50	9.7	13.8	NS	121.3
(Range)	(7.1 - 12.8)	(9.9 - 17.6)		(94.8 - 150.6)
LD-70	19.3	19.9	NS	171.0
(Range)	(14.5 - 27.5)	(15.6 - 27.1)		(139.0 - 242.8)
LD-90	52.6	33.5	NS	280.7
(Range)	(35.4 - 97.1)	(25.1 - 60.9)		(208.4 - 560.3)

TABLE 7

SODIUM BISULFITE
CHI-SQUARE ANALYSES of ABNORMALITIES

Dose Level, mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
1.0	-	-	-	0.19
2.0	2.05	-	-	-
4.0	6.68	-	-	0.18
5.0	0.0	0.0	0.50	-
10.0	0.0	0.0	0.49	-
20.0	0.0	0.0	-	-
25.0	0.0	0.0	0.0	-
30.0	-	0.0	-	-
40.0	-	0.0	-	0.18
50.0	2.88	-	-	-
100.0	0.0	0.0	0.0	0.0
200.0	0.0	-	0.0	0.19
All Doses (DF)	35.75 (9)	0.0 (7)	3.42 (5)	2.16 (5)

TABLE 8

SODIUM BISULFITE
CHI-SQUARE ANALYSES of H-L-S-V ABNORMALITIES

Dose Level, mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
1.0	-	-	-	0.19
2.0	2.05	-	-	-
4.0	4.31	-	-	0.18
5.0	0.0	0.0	0.50	-
10.0	0.0	0.0	0.49	-
20.0	0.0	0.0	-	-
25.0	0.0	0.0	0.0	-
30.0	-	0.0	-	-
40.0	-	0.0	-	0.18
50.0	2.88	-	-	-
100.0	0.0	0.0	0.0	0.19
200.0	0.0	-	0.0	0.19
All Doses (DF)	30.16 (9)	0.0 (7)	3.10 (5)	2.16 (5)

Table 9

SODIUM BISULFITE
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Untreated Controls		7	1	celosomia
			1	microphthalmia-lf., anopthalmia-rt.
			1	exencephaly, agenesis-maxilla
			2	dwarfism
			1	cachexia, anophthalmia - bilateral
			1	cachexia
Drilled Control - 0 hrs		0	0	
Drilled Control - 96 hrs		1	1	anophthalmia-rt., exencephaly, dysgnathia
Pierced Control - 0 hrs		0	0	
Pierced Control - 96 hrs		2	1	exencephaly
			1	anophthalmia-bilateral, dysgnathia
In Water-Air Cell - 0 hrs				
200.0 mg/kg		0	0	
100.0				
50.0	18	1	1	thoracopagus
25.0		0	0	
20.0		0	0	
10.0		0	0	
5.0		0	0	
4.0	99	4	1	shortened maxilla
			1	hemorrhage - umbilical cord
			1	celosomia
			1	agenesis - maxilla
2.0	99	2	1	malrotation-rt. ankle
			1	anophthalmia - bilateral, exencephaly, agenesis

Table 9 cont'd
(sheet 2 of 3 sheets)

SODIUM BISULFITE
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In Water - Air Cell - 96 hrs				
100.0 mg/kg	59	0	0	
40.0		0	0	
30.0		0	0	
25.0	56	0	0	
20.0		0	0	
10.0		0	0	
5.0		0	0	
0.0		0	0	
0.0		1	1	malformation - head

Table 9 cont'd
(sheet 3 of 3 sheets)

SODIUM BISULFITE
TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
In Water - Yolk - 0 hrs				
200.0 mg/kg		0	0	
100.0		0	0	
25.0		2	1	dwarfism
			1	malposition
10.0		1	1	anophthalmia-left, shortened- maxilla, flexion-mandible, phocomelia-lf. wing, micromelia-rt. wing, micromelia-rt. hind limb, ectrosyndactyly-lf. toe
5.0		1	1	exencephaly
In Water - Yolk - 96 hrs				
200.0 mg/kg		0	0	
100.0		0	0	
40.0		0	0	
4.0		0	0	
1.0		0	0	
0.0		1	1	anophthalmia-rt.

TABLE 10

SODIUM BISULFITE
POST HATCH DATA

Incubation Date 12/27/71

Label	Dose mg/kg	Age at Sexual Maturity, Days	Average Body Wt., gm at Hatch	Average Body Wt., gm 6 wks M	Average Body Wt., gm 6 wks F	Average Body Wt., gm 6 mos M	Average Body Wt., gm 6 mos F	Average Feed Consumption per bird 6 wks gm	Average Feed Consumption per bird 6 mo kg
210	10.0	145	40.3	408	371	1643	1476	988	11.1
211	5.0	148	38.5	456	370	1643	1589	965	11.1
212	Water	142	40.1	432	397	1702	1589	997	11.1